

AMENDMENT

Please amend the application as follows:

IN THE SPECIFICATION:

Please replace the paragraph that spans page 4, line 15, to page 5, line 10, with the following paragraph:

B²

Recently, we have successfully identified an anti-MDM2 antisense PS-oligo that effectively inhibits MDM2 expression in tumor cells containing MDM2 gene amplifications (Chen L. et al., *Proc Natl Acad Sci USA*, **95**: 195-200, 1998). Effective anti-human-MDM2 antisense PS-oligos were initially screened in two cell lines, JAR (choriocarcinoma) and SJSA (osteosarcoma), that contain wild type p53, amplified MDM2 genes, and overexpression of MDM2 oncoprotein. Of nine PS-oligonucleotides screened, Oligo AS5 (5'-GATCACTCCCACCTTCAAGG-3'; SEQ ID NO:28), which can hybridize to a position ~360 bp downstream of the translation start codon, was found to reproducibly decrease MDM2 protein levels in both cell lines by 3-5 fold at concentrations of 100-400 nM in the presence of Lipofectin. The mismatched control Oligo M4 (5'-GATGACTCACCACCATCATGG-3'; SEQ ID NO:5) had no effect on MDM2 expression. Oligo AS5 was also shown to induce RNase H cleavage of the target MDM2 mRNA, resulting in truncation and degradation of the target. Further studies demonstrated that, following AS5 treatment, the p53 protein level was elevated and its activity was increased. A dose-dependent induction of p21 expression by AS5 was observed up to 6.6 fold at the optimal concentration of 200 nM, suggesting that p53 transcriptional activity be increased following inhibition of MDM2 expression. JAR cells treated with AS5 showed a significant increase in the levels of apoptosis. AS5 did not cause visible apoptosis in the H1299 cells that lack p53. These results suggested that apoptosis induced by AS5 is due to activation of p53 following MDM2 inhibition by the oligonucleotide.

Please replace the paragraph that spans page 6, lines 10-20, with the following paragraph:

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p53-independent activity of MDM2 has been suggested by several reports and reviews. MDM2 gene products include several forms of polypeptide, representing alternatively spliced MDM2 variants. Various alternatively spliced MDM2 polypeptides have been found in several human tumors. Of the five forms of MDM2 analogs, only one retains p53 binding capability. However, cDNAs coding for all five forms of alternatively spliced MDM2 could independently transform NIH3T3 cells, indicating that these MDM2 transcripts have the p53-independent transforming ability. The effects of MDM2 overexpression on mammary tumorigenicity are seen in p53-null mice, indicating that MDM2 can cause transformation and tumor formation via a p53-independent mechanism. More recently, overexpression of MDM2 is shown to be associated with resistance to the antiproliferative effects of transforming growth factor β (TGF- β), which is p53-independent.

Please replace the paragraph that spans page 10, line 8, to page 11, line 3, with the following paragraph:

B³

In a fifth aspect, the invention provides *in vitro* and *in vivo* models to evaluate the therapeutic effectiveness of a recently identified anti-human-MDM2 antisense oligonucleotide (Chen L et al., *Mol Med* 5: 21-34, 1999; Wang H. et al., *Int J. Oncol.* 15: 653-660, 1999) in the treatment of human colorectal cancers when administered alone or in combination with conventional chemotherapeutic agents. Specifically, the primary goals are: 1) to obtain new oligos with better *in vivo* stability that can be used in future *in vivo* studies; 2) to determine the effects of anti-MDM2 oligos on human tumor cells with varying status of p53 and/or MDM2 expression; and 3) to identify the candidate cell lines that can be used in future *in vivo* studies. PS-oligonucleotide AS5-2 (5'TGACACCTGTTCTCACTCAC-3'; SEQ ID NO:36) was shown to have the highest activity in tested cell lines and was used in further studies. Thus far, 26 cell lines (16 types of human cancers) have been tested with AS5-2 in comparison with control oligonucleotides. Oligo AS5-2 significantly activated p53 activity in all cells with low levels of wild type p53, even in those with very a low level of mdm2 expression (Chen L. et al., *Mol Med* 5: 21-34, 1999). AS5-2 has no effect on p53 levels in cells with null p53, H1299 and SK-N-MC, or those with mutant p53. Based on the above screening, a modified analog of AS5-2 with advanced antisense chemistry, Oligo AS, was designed and evaluated in subsequent studies. In

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cell lines that contain wild type p53 and amplified MDM2 gene, SJSA and JAR, Oligo AS specifically inhibits MDM2 expression and p53 levels are elevated accordingly (Wang H. et al., *Int J. Oncol.* 15: 653-660, 1999).

Please replace the paragraph that spans page 21, lines 7-11, with the following paragraph:

B⁴
In certain preferred embodiments, these internucleoside linkages may be phosphodiester, phosphotriester, phosphorothioate, or phosphoramidate linkages, or combinations thereof. The term oligonucleotide also encompasses such polymers having chemically modified bases or sugars and/or having additional substituents, including without limitation lipophilic groups, intercalating agents, diamines and adamantane.

Please replace the paragraph that spans page 22, lines 14-21, with the following paragraph:

B⁵
For purposes of the invention, a "hybrid oligonucleotide" refers to an oligonucleotide having more than one type of nucleoside. One preferred embodiment of such a hybrid oligonucleotide comprises a ribonucleotide or 2'-O-substituted ribonucleotide region, preferably comprising from about 2 to about 12 2'-O-substituted nucleotides, and a deoxyribonucleotide region. Preferably, such a hybrid oligonucleotide will contain at least three consecutive deoxyribonucleosides and will also contain ribonucleosides, 2'-O-substituted ribonucleosides, or combinations thereof. Examples of such hybrid oligonucleotides are disclosed in U.S. Patent Nos. 5,652,355 and 5,652,356.

Please delete the paragraph that spans page 29, lines 4-9.

Please replace the paragraph that spans page 47, line 18, to page 48, line 5, with the following paragraph:

B⁶
Test Oligonucleotides. The test oligonucleotide, Oligo AS, a 20-mer mixed-backbone oligonucleotide (5'-UGACACCTGTTCTCACUCAC-3'; SEQ ID NO:47) and its mismatched